



MONTANA DEPT. OF LIVESTOCK
ANIMAL HEALTH DIVISION
P.O. BOX 202001
HELENA, MT 59620-2001
406-444-2043



USDA, APHIS, VS
208 N. MONTANA AVE, SUITE 101
HELENA, MT 59601
406-449-2220

February 28, 2005

Dear Montana Sheep Producer and Accredited Montana Veterinarian:

The Montana Department of Livestock (DOL) received USDA-APHIS cooperative agreement funding again this year for scrapie eradication efforts. It is the intent of the DOL, and supported by the Montana Scrapie Advisory Committee, to make a portion of the funds available to sheep producers to perform official genotype testing. Regarding genotype testing, one of the conditions of the cooperative agreement is that **funds will only be available for testing black-faced breeds of sheep**. Additionally, the funds may be used for 3rd eyelid testing, scrapie suspect testing, scrapie slaughter surveillance, and scrapie educational efforts in Montana. These funds will be available for these scrapie eradication-related activities **completed by September 9, 2005**.

OFFICIAL GENOTYPE TESTING (Black-faced breeds of sheep):

- Please be reminded- this is not intended for regulatory purposes, rather it is a tool for producers to make flock management decisions regarding flock / animal genetic susceptibility to scrapie.
- Cost of official genotype testing (laboratory cost only) for up to 150 animals per producer. Producer is responsible for attending veterinarian's fees (professional services, mileage, etc.) The official genotype testing may include:
 - Testing 150 animals at Codon 171;
 - Testing 75 animals at Codon 171 and at Codon 136; or
 - Testing 150 animals at Codon 136 (if animals have already been officially tested at Codon 171).

Additional information regarding Scrapie Cooperative Agreement Funding - the following must be met to qualify as official testing and be eligible for funding:

- An accredited veterinarian must collect Blood samples or other authorized state or federal animal health official. Blood samples: minimum of 2 cc whole blood in EDTA tube (purple top).
- Each sheep tested **must be officially identified with approved scrapie program ID device**.
- The blood sample must be submitted with a properly completed VS Form 5-29. The VS Form 5-29 MUST be an original with an APHIS serial number. This form is available to accredited veterinarians from the USDA-APHIS-VS Helena office (406-449-2220). A sample Form 5-29 is attached. **These forms must be filled out completely and legibly and must include the official identification of each sheep tested.** Please allow ample time for the forms to be sent to your veterinarian's office.
- The CERTIFICATION FOR PAYMENT Block of the VS Form 5-29 should be completed as follows: The submitting veterinarian should check the "**at State/Federal Expense**" box. Also, enter the code "**CO-OP**" which identifies the test as a cooperative agreement to the left of the "at State/Federal Expense" box and indicate on the form:

Invoice to: Montana Department of Livestock
P.O. Box 202001
Helena, MT 59620-2001
- The "CODON TESTING" box in the TYPE TEST Block of the VS Form 5-29 should be checked, and indicate which Codon is to be tested (Codon 171, Codon 136, or both Codons 171 and 136).
- The genotyping laboratory has been approved by APHIS (approved laboratory list attached).
- A copy of the official results will be provided to USDA-APHIS-VS to be entered in the Scrapie National Generic Database (SNGD). Official results will be sent to the Department of Livestock for distribution to USDA-APHIS-VS and to the submitting veterinarian.
- Funds available for official genotype testing of blood samples collected and submitted from **September 10, 2004 through September 9, 2005**. The funds will be allocated on a "first come, first serve" basis

OFFICIAL 3RD EYELID TESTING (Black faced breeds of sheep)

- All costs associated with official 3rd eyelid testing of **20 animals per producer**.
- Animals must have been previously official genotype tested as **QQ at Codon 171** to be eligible.
- Animals must be **older than 14 months** of age to be eligible.
- An authorized state or federal animal health official must collect 3rd eyelid samples.
- Please contact the USDA-APHIS-VS office to schedule appointment (406-449-2220).

SCRAPIE SLAUGHTER SURVEILLANCE

- All costs associated with official scrapie testing (brain tissue) of **5 animals per producer**.
- Animals must be **older than 14 months of age** to be eligible.
- Animals slaughtered on-ranch or at a state or federal inspected facility are eligible.
- An authorized state or federal animal health official or an approved accredited veterinarian must collect appropriate brain tissue samples.
- Please contact the USDA-APHIS-VS office (406-449-2220) or the Department of Livestock (406-444-2043) to schedule appointment.

SCRAPIE EDUCATIONAL EFFORTS

- Please contact the Montana Department of Livestock for scrapie-related educational materials or to schedule seminars for livestock producer meetings, local 4-H or FFA meetings, etc. (406-444-2043).

Again, the cooperative agreement funds will be available for the above scrapie eradication efforts from September 10, 2004 through September 9, 2005. Thank you for your interest and participation in helping to eradicate scrapie.

For more details regarding this project, please contact:

- Dr. Thomas F.T. Linfield, Department of Livestock at 406-444-2043 or tlinfield@mt.gov OR
- Dr. Jeanne Rankin, Department of Livestock at 406-444-2043 or jrankin@mt.gov OR
- Becky Frey, USDA-APHIS-VS, at 406-587-2864 or Rebecca.K.Frey@aphis.usda.gov
- Rodney Kott, MSU Extension Service, at 406-994-3415 or rkott@montana.edu

Approved Genotyping Laboratories

Biogenetic Services, Inc. 801, 32 nd Avenue Brookings, SD 57006 Contact: Alex Kahler Phone: 605-697-8500 Fax: 605-697-8507 Web: www.biogeneticservices.com	HAP Typing Facility Genaissance Pharmaceuticals Five Science Park New Haven, CT 06511 Contact: Jeffrey M. Otto, Ph.D Phone: 203-786-3546 Fax: 203-786-3699 Web: www.genaissance.com	GeneSeek, Inc. 4711 Innovation Dr. Lincoln, NE 68521 Contact: Mr. Abe Oommen Phone: 402-435-0665 Fax: 402-435-0664 Web: www.geneseek.com
BioServe Biotechnologies, Ltd. 1050 West Street Laurel, MD 20707 Contact: Dr. Terri Lehman Phone: 301-470-3362 Fax: 301-470-2333 Web: www.bioserve.com	GeneCheck, Inc. 1629 Blue Spruce Drive Suite 106 Ft. Collins, CO 80524 Contact: Ms. Tresea Mulqueen Phone: 800-822-6740 Fax: 970-472-9956 Web: www.genecheck.com	Veterinary Genetics Laboratory School of Veterinary Medicine University of California One Shields Avenue Davis, CA 95616-8744 Contact: Customer Service Phone: 530-752-7383 Fax: 530-752-3556 Web: www.vgl.ucdavis.edu
Diagnostic Center for Population and Animal Health Michigan State University Room A45, Veterinary Medical Center E. Lansing, MI 48824 Contact: Roger Maes, DVM, PhD Annabel Wise, DVM, PhD Mr. Dan Taylor, MS Phone: 517-353-2296 Fax: 517-353-4426 Web: www.dcpah.msu.edu	GenMARK (Vita-Tech Laboratories, LLC) 1825 Infinity Drive DeForest, WI 53532 Contact: Ms. Lynette Childs Phone: 608-846-0580 Toll-Free: 877-766-3446 Fax: 608-846-0584 Web: www.genmarkag.com T	

The Genetics of Scrapie Susceptibility

Scrapie is an infectious disease; a susceptible animal must come in contact with the disease agent to become infected. Scrapie does not occur in any sheep of any genotype that has not been exposed to the infectious agent. However, once exposed to the agent, the genotype of the animal has a profound effect on which sheep may become infected and eventually die. The interaction between the scrapie agent and host genetics is not fully understood. The following is a summary of the current knowledge. It is possible that additional genes or sites on the prion gene will be identified that will also impact susceptibility.

General Background

To understand the genetic component of scrapie, it is necessary to review some of the basics of molecular genetics. In the 1950's Watson and Crick discovered that the genetic code was contained in the double helix molecule of deoxyribonucleic acid (DNA). The basic unit of DNA consists of three chemical elements (a nitrogenous base, a phosphate group, and a deoxyribose sugar molecule). This unit is known as a nucleotide. DNA is present in all nucleated cells of the body and is passed to subsequent generations in the eggs and sperm of mammalian species. Subsequently, it was discovered that DNA encodes proteins. Proteins are composed of amino acid chains. Scientists have determined that DNA codes the amino acid sequence of proteins through sets of three nucleotide bases. Each set of three

nucleotide bases is called a codon; each codon codes for one amino acid. Chromosomes are made up of DNA strands. Chromosomes always occur in pairs, one from the sire and one from the dam.

The two chromosomes that make up a chromosome pair code for the same proteins. The amino acid sequence that makes up a specific protein usually remains constant from generation to generation and from animal to animal within a species. Rarely, a change occurs at a codon site resulting in a different amino acid sequence; this variability in amino acid sequence is known as polymorphism. In most instances, polymorphism is thought to have little effect on the resulting protein produced. However, in the case of the normal prion protein (PrP cellular), polymorphism can have a profound effect on scrapie susceptibility.

All animals that have been studied have a gene that codes for the normal prion protein. The function of normally occurring cellular prion protein is unknown.

Understanding the Scrapie Agent's Interaction with the Host Genotype

The current state of knowledge about what causes scrapie must be examined to understand how different codons influence susceptibility to scrapie. Various causes of scrapie have been theorized; however, a majority of scientists believe that the causative agent is an abnormal form of a normally occurring cellular prion protein known as PrP scrapie. PrP cellular, the normally occurring cellular prion protein, is found in all tissues that have been examined. Stanley Prusiner received the Nobel Prize in 1998 for his work supporting this theory. The basis of this theory is that an abnormally conformed prion protein, PrP scrapie, serves as a template to influence a geometrical conformation change in the normal PrP cellular produced by the exposed animal. This abnormal protein (PrP scrapie) accumulates. After a period of months and more often years, it causes nervous system dysfunction and, eventually, the death of the animal. The abnormal prion proteins (PrP scrapie) may be found in the nervous system, the spleen, lymph nodes, placenta, intestine, blood, pancreas, ovary, and liver of infected sheep.

The gene that encodes the normal prion protein has polymorphisms at codons 136, 154, and 171 that influence the ability of the prion cellular protein structure to be geometrically altered by the PrP scrapie template when the animal is exposed to it. At this time, no such polymorphisms have been identified for goats. All goats, therefore, must be assumed to be susceptible.

Genetic Susceptibility to Scrapie

- Codon 136 codes for either the amino acid valine (V) or alanine (A);
- Codon 154 codes for either histidine (H) or arginine (R); and
 - Codon 154 plays a minor role in scrapie susceptibility and is not often used in the United States. Codon 154 is not a consideration in the US Scrapie Eradication Program at this time.
- Codon 171 codes for glutamine (Q), arginine (R), lysine (K), or histidine (H).
 - The presence of H at 171 is presently thought to be equivalent to Q for scrapie resistance. K at 171 has recently been found in a few Barbados sheep; its effect on scrapie resistance has not been studied.

US sheep have 3 major forms (alleles) of the scrapie susceptibility gene: AQ, AR, and VQ; and 2 minor forms: AH and AK. The VQ allele occurs at a significantly lower frequency than AQ or AR. For the purpose of this discussion H or K at 171 will be considered equivalent to Q. Each sheep inherits two copies of each gene and thus two alleles (one from each parent). Codons 136 and 171 are close together on the same chromosome so the offspring will always receive one of the alleles of each parent and not a mixture of the two. In the United States, codon 171 appears to be the major determinant of relative scrapie susceptibility. In some flocks, codon 136 may also play a role.

Each gene has a pair of alleles, one on each chromosome of a chromosome pair. Alleles reside in the same site on each chromosome. When only codons 171 and 136 are considered and H or K at 171 is treated as a Q at 171, there are only four combinations that need to be considered in order to eliminate scrapie from a flock: AARR, AAQR, AVQR, and QQ.

- 1) AA RR sheep are nearly completely resistance to scrapie. Only one case (in Japan) has ever been reported. These sheep are highly unlikely to carry or transmit scrapie;
- 2) AA QR sheep are rarely susceptible. In rare cases, AA QR sheep in Europe have become infected. Most but not all cases have been in flocks with high scrapie prevalence. It is unknown whether infected AA QR sheep can transmit the disease. The risk from exposed AA QR sheep is probably minor, since infected AA QR sheep are rare and it is unusual for PrP scrapie to be found outside the brain of these sheep;
- 3) AV QR sheep are somewhat susceptible to some scrapie strains. Two cases have been identified in the US. The risk from exposed AV QR sheep is probably minor, since infected AV QR sheep are rare and it is unusual for PrP scrapie to be found outside the brain of these sheep. AV QR sheep are significantly less susceptible to the scrapie strains that affect them than are the QQ sheep that are affected by these strains;
- 4) QQ Sheep (AA QQ, AV QQ, and VV QQ) are susceptible to scrapie and can transmit the disease to susceptible flock mates.

Genetics as a Tool for Eradicating Scrapie

(This section would only apply in the event a flock is determined to be an infected flock)

Genetic selection is being used as the primary means of scrapie control in the Netherlands and the United Kingdom. In the United States, the U.S. Department of Agriculture's (USDA) Animal Plant Health Inspection Service (APHIS) is using genetic testing to determine which exposed animals must be removed or restricted in affected flocks and which are free to move unrestricted. APHIS policy recognizes the importance of codon 171 and the potential importance of codon 136 in the transmission of scrapie in the United States.

The National Genetics Based Flock Clean-up Plan allows affected producers to retain or move RR sheep, AA QR sheep, and most AV QR sheep without restriction. It also calls for the removal or restriction of all exposed QQ ewes, exposed female goats, and the female offspring of scrapie positive female animals. In a minority of flocks where positive AV QR sheep are identified, exposed AV QR ewes will be removed or restricted. In other flocks, when requested by the owner, APHIS will remove exposed AV QR ewes for study. All scrapie positive and suspect animals must be removed. In the unlikely event that scrapie is found in a sheep that is neither QQ nor AV QR, additional animals may be required to be removed or restricted.

In a small percentage of flocks that are either heavily infected, that have cleaned up and then had a recurrence of scrapie in animals born on the premises, or where the epidemiology is different from that seen in most flocks, additional animals may be purchased for study and/or additional restrictions may be placed on the flock.

Owners of affected flocks that comply with the requirements of the National Genetics Based Flock Clean-up Plan are eligible for indemnification of any animals that are removed as part of the flock plan in accordance with Title 9 Code of Federal Regulations, part 54. Flocks whose owners do not retain restricted female animals will not be considered exposed flocks once they have completed the flock cleanup plan. If an owner elects to retain restricted female animals, additional restrictions will be placed on the flock. These flocks will be considered exposed flocks until all such animals are removed or a five- year monitoring plan is completed.

Other Tools for Eradicating Scrapie

In addition to a genetics based flock clean up plan, USDA is using several tools to eradicate scrapie. These include (1) finding infected and source flocks through the testing of exposed animals traced out of known infected flocks and, beginning in April 2003, through slaughter surveillance, (2) identification of sheep and goats in commerce to allow for effective tracing of scrapie positive and exposed animals, (3) restricting the movement of genetically susceptible exposed animals, and (4) educating producers, veterinarians, and others about clinical signs of scrapie.